ALFALFA BLOTCH LEAFMINER (DIPTERA: AGROMYZIDAE) LABORATORY STUDIES OF BIOLOGY IN EUROPE[1]

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ABSTRACT—Females (1 to 2 day-old) of the alfalfa blotch leafminer, *Agromyza frontella* (Rondani), an introduced agromyzid that is a serious pest on alfalfa in the eastern United States oviposited readily in the laboratory on the under surface of alfalfa leaflets. The newly deposited eggs measured 0.35 mm long, and eggs hatched 5–6 days after oviposition. Larvae cut their way out of mines 5–6 days after the mines became visible. The cycle from egg to adult emergence required about 33 days. In France, parasitism of field-collected *A. frontella* larvae by diverse species often ranged from 70 to 90%. Several of these parasitoid species are being considered for introduction into the US as natural control agents against *A. frontella*.

From the responses to a questionnaire sent to entomology cooperators in 18 eastern states it has been determined that Massachusetts constantly suffers the most severe damage from the alfalfa blotch leafminer, *Agromyza frontella* (Rondani). However, in Bershire County, Connecticut, on June 14, 1971, 58% of the alfalfa leaflets were damaged by *A. frontella* larvae. Also, in 1969–70, *Agromyza* sp., probably *A. frontella*, caused a loss of about 10% of the alfalfa crop, a pecuniary loss of about $9/acre involving the first and second cuttings in Connecticut (Anonymous, 1972).

Although *A. frontella* is widespread in Europe (Spencer, 1973), it is not economically important there; perhaps because it is held in check by natural enemies. Therefore, since April 1973, the European Parasite Laboratory has been engaged in field and laboratory studies of the agromyzids *A. frontella*, *A. nana* Meigen and *Liriomyza congesta* (Becker) and their natural enemies. Total parasitism of *Agromyza frontella* by the diverse parasitoids frequently ranged from 70 to 90%. Several of these parasitoids are being considered for introduction into the United States as biocontrol agents against this pest. To date, the following species of parasitoids of the agromyzids, *A. frontella*, *A. nana* and *Liriomyza congesta*, have been recovered during the studies in Europe: (Braconidae) *Opius dureseani* Fischer, *O. propodealis* Fischer, *Phanomeris braconius* (Haliday); (Eulophidae) *Chrysocharis* ? *nitetus* (Walker), *Cirrospilus vittatus* Walker, *Dacnusa dryas* (Nixon), *Diglyphus isaea* (Walker), *Pnigalio* sp.,

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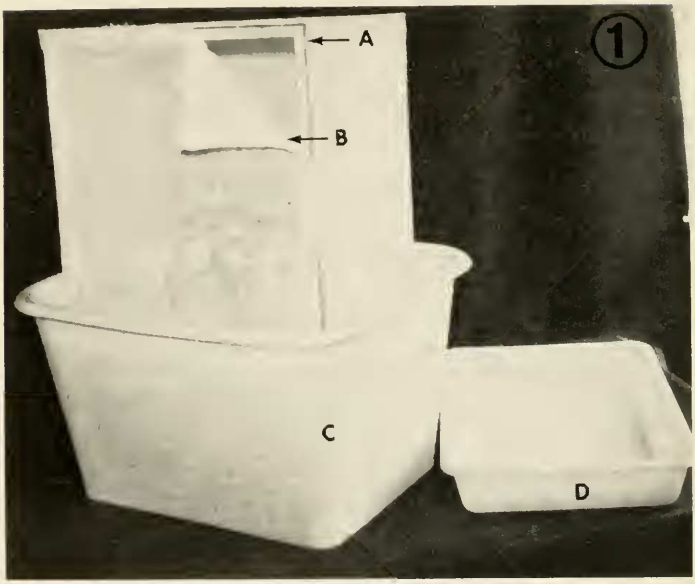

Fig. 1. Rearing cage. A, hook-equipped side of Velcro strip. B, eye-equipped side of Velcro strip. C, large cage bottom. D, small cage bottom.

"*Tetrastichus*" sp.; (Pteromalidae) *Miscogaster* ? *masculata*, ?*M.* sp., *Systasis* sp. and ?*Trichomalus*.

Two ectoparasitoids, *Phanomeris braconius* and *Diglyphus isaea*, and the endoparasitoids, *Opius propodealis* and *Dacnusa dryas*, have all been reared through several generations on *Agromyza frontella*. *Phanomeris braconius* was released in the US in the spring of 1974. *Diglyphus isaea* and *Opius dureseaui*, a new species (Fischer, 1975), are presently being reared at the USDA Beneficial Insects Research Laboratory, Newark, Delaware, for spring and summer release in 1975–76. The taxonomy of *Agromyza frontella* was studied by Steyskal (1972) and Spencer (1973). However, little was known about the biology of the pest. The present paper summarizes the results of the laboratory studies made at the European Parasite Laboratory on the biology of this pest.

Materials and Methods

The basic cage used in rearing *A. frontella* was a rectangular frame (40 × 25 × 42 cm) of wooden strips (1.5 × 1.5 cm) covered on 5 sides with fine mesh Velcro® attached to the strips with neoprene base glue. Access to the cage was provided by sewing the eye-equipped side of a strip of Velcro® to 2 or more edges of 1 face of the frame and gluing the hook-equipped strip to the

appropriate wooden strips (Fig. 1A, 1B). Two sizes of plastic containers (ID 50 × 38 × 29 cm, and 45 × 30 × 6.5 cm) held the alfalfa plants and served as the cage bottom (Fig. 1C, 1D).

Alfalfa plants (Europe variety) used in the study were grown in the laboratory in terra cotta pots (diam 16 × 22 cm) in a mixture of 50% garden soil, 25% peat, and 25% vermiculite. In tests requiring a self-sustaining ecosystem throughout the test period, the posts were placed in the large containers. For convenience, when such an environment was not necessary, the pots were placed in the small containers. Vermiculite was poured into the container until it was level with the upper edge of the terra cotta pots. A layer of sifted, fine, white sand was then poured onto the vermiculite and the soil of the plant to a depth of about 1.5 cm to retain moisture and to facilitate the recovery of dead flies at the end of a test period.

The assembled cage containing insects was placed inside a semi-programmed test chamber maintained at 22.2 + 1.5°C and 60 + 5% RH during the 18-h photophase and at 17 + 0°C and 65 + 5% during the 6-h scotophase. Between 0830 and 1700 h each day at about 2-h intervals, the temperature was first lowered and then raised by 3°C for 15 min. Also, the cages were rotated 90° at 2-h intervals. An oscillating fan (height 50 cm and diameter 30 cm) was placed about 1 m from the cages to agitate the alfalfa leaflets.

Four to 5 days after the 1st mines became visible on alfalfa leaflets, mined leaflets were removed and placed in individual petri dishes (50 × 12 cm) on slightly moist sand. However, field observations subsequently showed that mature fly larvae placed in plastic bags often left the mines immediately if the temperature was raised 4–5°C above ambient and formed puparia within an hour after cutting out of the mines. Therefore, mined leaflets were removed the 6th day after mines became visible and were placed in transparent plastic bags. The mature larvae and puparia that were recovered in this way were placed in petri dishes in groups of 10. The petri dishes were placed in the control chamber and held for adult emergence.

For the oviposition and longevity studies, the adult flies were put into pint cardboard cartons with bottoms of nylon cloth, and which contained a pipe cleaner support (Fig. 2). Honey was dabbed onto the nylon cloth and a moistened sponge covered with a petri dish was placed over the nylon. Also a pipe cleaner dabbed with droplets of honey and moistened was inside the cartons. The cartons were held at room temperature, placed in the control chamber or held in a refrigerator at 4.4–7°C.

Results

Agromyza frontella females from one to ten days old oviposited immediately when they were exposed to alfalfa. The ovipositor was inserted a short distance into the under surface of a leaflet without puncturing the upper surface. The deposited egg (0.35 mm long, Fig. 3) was usually found toward the base and near a border. Oviposition scars were smaller and less easily seen than scars caused by adult feeding; though both kinds of scars were made with the ovipositor.

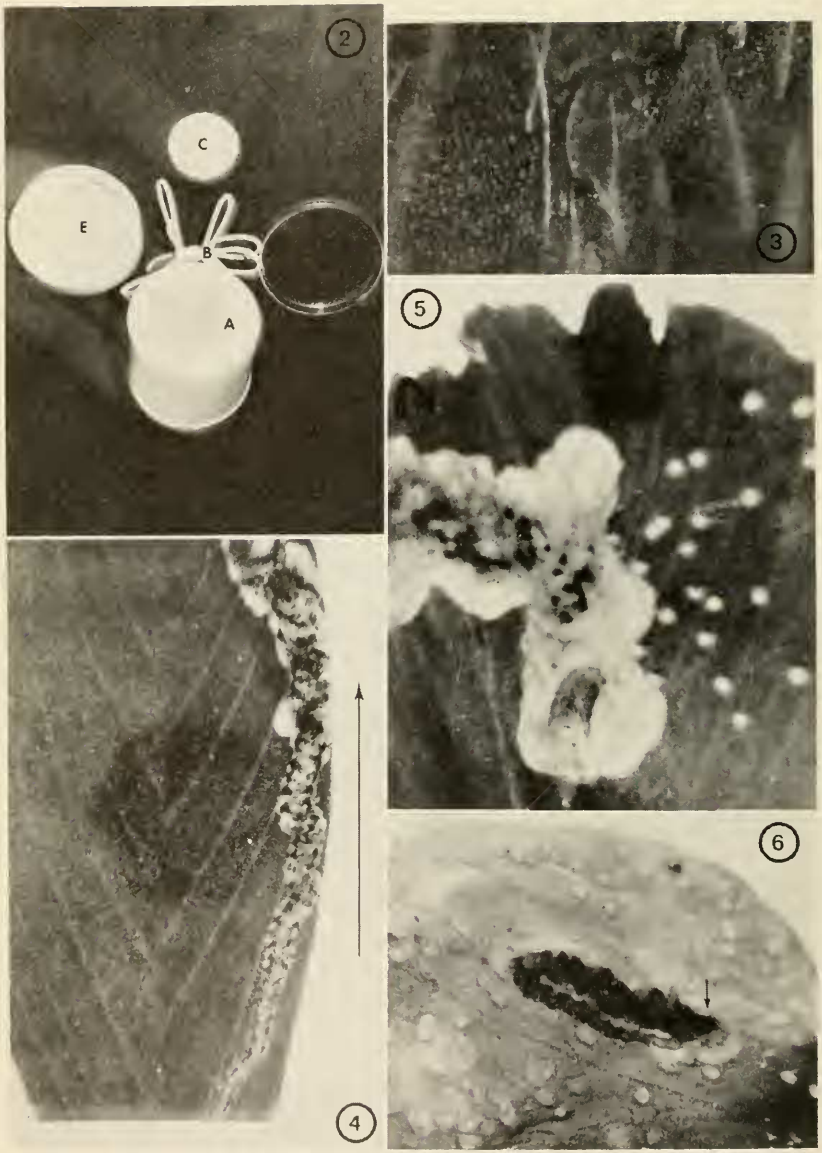

Fig. 2. Pint cardboard carton used for oviposition and longevity studies. A, tergal cloth bottom. B, pipe cleaner. C, sponge. D, petri dish. E, top of cardboard carton. Fig. 3. Egg of *Agromyza frontella*, 0.35 mm long, inside mine, on under surface of alfalfa leaflet. Fig. 4. Early stage of typical *A. frontella* mine. Arrow indicates direction of mining from base toward apex of alfalfa leaflet. Fig. 5. Late stage of typical *A. frontella* mine. Note blotch in center of leaflet and the mature larva within the mine. Fig. 6. Dead *A. frontella* larva inside mine on leaflet fed on heavily. Note bleached area surrounding dead larva.

At our laboratory conditions, the eggs hatched five to six days after oviposition. Newly emerged larvae always began mining from the point of oviposition toward the leaf border (Fig. 4), continued along the border of the leaf and toward the apex and then turned inward toward the center. The mine was broadened gradually until both sides of the center vein of the leaflets were consumed (Fig. 5). The larvae cut their way out of mines five to six days after the mines became visible. Within one to several hours, the cuticle of the mature larvae shrank in length, hardened and rounded to form typical cyclorrhaphous, dipterous puparia. An additional 20–23 days were required for larval/pupal metamorphosis and adult emergence in the laboratory.

Adult flies emerged from the puparia by splitting them along lines of weakness situated laterally on the cephalic extremity. Shortly after emergence the adult flies distended their wings and darkened in color. If water and honey were available immediately after emergence, flies kept in pint cartons at 4.4–7°C lived as long as 15 days if the cartons were removed from the refrigerator each day, dabbed with honey, sprayed with water from an atomizer, and held at room temperature for 1 h. The longevity of flies in the rearing cages did not differ significantly from the longevity of flies kept in the oviposition cartons.

In cages with high populations (40 flies/400–500 leaflets), adult flies often destroyed entire plants by puncturing the leaflets with the ovipositor and feeding on the juice. As a result, the leaflets became brittle and bleached. Hasan (1971) reported similar damage caused by field populations of a carrot miner *Napomyza carotae* Spencer, feeding on carrot leaves in North Wurttemberg, West Germany. The *Agromyza frontella* larvae were never able to complete their development in areas of alfalfa leaflets that were fed on heavily by the adults (Fig. 6).

No mating was observed during our studies though males and females of varying ages were observed in pint cardboard cartons, glass tubes and oviposition cages on numerous occasions for as long as a half hour. Nevertheless, after five generations, the ratio of females to males remained constant at about 1:1 in the subsamples examined. I assume, therefore, that mating occurred in the oviposition cages on the underside of the slightly curled alfalfa leaflets. In addition, from the number of oviposition scars and the ratio of females to males in several tests, both oviposition and mating were stimulated by alternately raising and lowering the temperature four times a day and by continuously agitating the alfalfa leaflets by the air current from the oscillating fan. The frequencies of oviposition scars and mating (based on sex ratio) were lower in cages not exposed to these conditions.

Discussion and Conclusion

Although this study showed that *A. frontella* is an acceptable host for the rearing of several species of natural enemies of agromyzids, the field bionomics of this pest and its natural enemies require further study in order to select and give priority to those parasitoid species most likely to reduce the damage caused by this pest in the US to a noneconomic level of importance.

Indicative of the need for further field studies is the fact that Spencer (1973) stated that no information on the rate of parasitism of this pest by its natural enemies was available. Of the four major parasitoids included in the present study, Spencer listed only *Dacnusa dryas* as having been recovered from *Agromyza frontella*. The number of field generations of *A. frontella* per year has not been clearly established. Spencer (1973) reported that there were at least two generations between June and September. However, in the Paris area, mature *A. frontella* larvae were collected as early as Apr. 10 in 1973 and 1974, and as late as Sept. 14 in Luxembourg and near Trier, Germany, in the same years. From the laboratory and field observations reported here, *A. frontella* in warm climates probably has from five to six generations/year.

Given the high percentage of parasitism by its natural enemies and its low level of economic importance in Europe, it seems likely that the importation into the United States of selected parasitoids will reduce considerably the damage caused by *A. frontella* in the eastern US.

Appreciation is expressed to John J. Drea of the European Parasite Laboratory, Agr. Res. Serv., USDA, Sèvres, France, for council during this study and for reading the manuscript. I also express appreciation to Roger W. Fuester of the same laboratory who read the manuscript and offered useful suggestions in its preparation.

Thanks are also due to the following for the identifications of hosts and parasitoids: Z. Boucek of the Commonwealth Institute of Entomology, London; D. C. Griffiths of the University of Alberta, Edmonton, Canada; P. M. Marsh, C. W. Sabrosky and G. C. Steyskal of the Systematic Entomology Laboratory, IIBIII, Agr. Res. Serv. USDA; and K. A. Spencer of the British Museum (Natural History), London. Very special appreciation is expressed to Max Fischer of the Naturhistorisches Museum, Vienna, Austria, who described the new species *Opius dureseaui*.

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